Effects of Subendocardial Ablation on Anodal Supernormal Excitation and Ventricular Vulnerability in Open-Chest Dogs

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Background. In Langendorff-perfused hearts and in hearts on cardiopulmonary bypass, chemical ablation of the subendocardium of both ventricles decreases ventricular vulnerability to fibrillation. It was hypothesized that the effects of ablation are a result of the elimination of the subendocardial Purkinje fiber network. This hypothesis has been supported by recent observations that the supernormal excitability that is demonstrable in the Purkinje fibers is associated with arrhythmogenesis.

Methods and Results. We tested this hypothesis on 10 open-chest dogs by evaluating the strength–interval curves of anodal and cathodal stimulation with the assistance of computerized mapping techniques. The ventricular fibrillation threshold was also determined. The same test was then performed after chemical ablation of the subendocardium of either the right ventricle (six dogs) or both ventricles (four dogs). Anodal supernormality was consistently demonstrated in all the dogs studied both before and after subendocardial ablation. The ventricular fibrillation thresholds were 23±5 mA both before and after right ventricular subendocardial ablation (p=NS). The ventricular fibrillation thresholds before and after biventricular subendocardial ablation were 25±3 and 22±10 mA, respectively (p=NS).

Conclusions. We conclude that 1) subendocardial ablation does not decrease ventricular vulnerability when the heart is in situ and is not on cardiopulmonary bypass and 2) anodal supernormal excitability can be demonstrated in ventricles without a subendocardial Purkinje fiber network. (Circulation 1993;87:216–229)

KEY WORDS • electrophysiology • Purkinje fibers • fibrillation, ventricular • Lugol's solution

The mechanism by which single premature electrical stimuli can induce ventricular fibrillation (VF) is unclear. However, several authors have proposed that the ventricular subendocardium is more important than other parts of the ventricle in the generation and maintenance of VF. Using transmural plunge electrode recordings, Worley et al demonstrated the development of an endocardial–epicardial gradient of activation rate within 2 minutes after the electrical induction of VF. Janse et al used Langendorff-perfused hearts to demonstrate that, after subendocardial ablation, the remaining epicardium can sus-

All editorial decisions for this article, including selection of reviewers and the final decision, were made by Bruce B. Lerman, MD, as guest editor. This procedure applies to all manuscripts with authors from the University of California San Diego or UCSD Medical Center.

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This work was done during the tenure of a Clinician Scientist Award (88 414) from the American Heart Association and was supported in part by the Department of Veterans Affairs, the Whitaker Foundation, the NIH FIRST award (HL-50259-01), and an AHA grant-in-aid (92009820) to P.-S.C.

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Received May 16, 1991; revision accepted September 22, 1992.

tain well-organized reentrant ventricular tachycardia but not VF. Damiano et al showed that, after chemical ablation of a thin layer of subendocardial fibers while the dog was on cardiopulmonary bypass, the VF threshold was greatly increased. In some animals, this treatment made VF not inducible. Because of these findings, it has been proposed that subendocardial ablation may become a potentially useful method in preventing the generation and the maintenance of VF in patients with primarily VF when the arrhythmia is refractory to medical therapy. The mechanism by which subendocardial ablation prevents the electrical induction of VF is unclear. Because Purkinje fibers are found only in the subendocardial tissue, however, it has been hypothesized that it is the elimination of the subendocardial Purkinje fibers that is responsible for decreased ventricular vulnerability. The direct association between Purkinje fibers and ventricular vulnerability has been further supported by the recent work of Chialvo et al. These authors demonstrated that, in sheep cardiac Purkinje fiber preparations, supernormal excitation can consistently be demonstrated by lowering the extracellular potassium chloride concentration. Experimental and numerical simulation results showed that, in the presence of this supernormal excitability and using pulses of appropriate strength, very complex nonmonotonic changes of activation can occur in these isolated Purkinje fibers. The authors proposed that these com-
plex activities demonstrated in the Purkinje fibers can lead to arrhythmia, which would explain the presence of a ventricular vulnerable period. Because other authors have previously demonstrated that supernormality is present only in the Purkinje fibers and not in the ventricular myocytes, chemical ablation of the subendocardial Purkinje fiber network may thus decrease or eliminate both supernormal excitation and ventricular vulnerability.

Before this hypothesis is accepted, however, further investigation on three matters is needed: 1) Previous studies were performed either when the heart was not in situ or when the dog was on cardiopulmonary bypass. The antifibrillatory effects of subendocardial ablation on a normally contracting heart have not been demonstrated. 2) The strength–interval curves were not determined in the previous studies of chemical ablation of the subendocardium in intact animals. Whether or not supernormal excitability is eliminated by subendocardial ablation is unknown. 3) If direct stimulation of the Purkinje fibers during the supernormal period is important in the generation of ventricular arrhythmia, it would follow that ablating only the Purkinje fibers near the site of stimulation should be sufficient to prevent the generation and the maintenance of VF. In previous studies, extensive subendocardial ablation of both ventricles was performed. It is thus not certain whether or not ablating only the subendocardium at the site of stimulation has the same efficacy in preventing VF.

The purpose of this study is to test whether or not chemical ablation of the canine subendocardium is associated with the abolition of both the Purkinje fibers and supernormal excitability. The results of our study will be used to test two hypotheses: 1) that chemical ablation of the subendocardium of both ventricles decreases ventricular vulnerability to VF in situ hearts not on cardiopulmonary bypass and 2) that the mechanism by which chemical ablation of the subendocardium decreases ventricular vulnerability is attributable to the elimination of supernormal excitability and the Purkinje fibers in the tissue near the site of premature stimulation.

**Methods**

**Protocol 1: Right Ventricular Subendocardial Ablation**

*Recording electrodes.* The recording electrodes were modified from the commercially available sock electrode harness (Bard Electrophysiology, Tewksbury, Mass.) to form six columns (columns A–F) and 8 rows (rows 1–8) of a recording electrode array with a 5-mm interelectrode distance (Figure 1A). An additional eight bipolar recording electrodes were positioned around the central portion of the array to allow more detailed recordings of the electrical events in that area. Thus, 56 electrodes were used. The distance between the two bipoles of each electrode was approximately 1 mm. One silver wire with a diameter of 0.125 mm, insulated except at the most distal 1–2 mm, was then threaded through the center. During the study, the uninsulated tip of the silver wire contacted the epicardium and served as the electrode for the cathodal unipolar baseline ($S_1$) stimulation, with the chest wall as ground. For the bipolar premature ($S_2$) stimulation, two silver wires, insulated except at the most distal 1–2 mm, were threaded through two of the four corners of the electrode array. One wire was used as the anode and the other as the cathode for both the VF threshold testing and the strength–interval curve determination. The two
Figure 2. Diagram showing the method of subendocardial ablation. Panel A shows the method of right ventricular subendocardial ablation. After the chest was opened and the heart suspended in a pericardial cradle, umbilical tapes were threaded around the venae cavae and the pulmonary artery. The two ends of the umbilical tape were then threaded through a plastic tube and were clamped with a hemostat. A hollow-lumen plastic catheter was then inserted via the right atrial appendage into the right ventricular apex. The catheter was connected to a syringe filled with normal saline or Lugold’s solution. During the study, both the inflow and the outflow of the right ventricle were occluded by pushing the plastic tube toward the ventricle cavity or the pulmonary artery, then clamping the tape with the hemostat. Normal saline or Lugold’s solution was injected into the right ventricular cavity for the control experiment and for the subendocardial ablation, respectively. The shaded area on the right ventricle indicates the location of the recording and pacing electrode array. IVC, inferior vena cava; LA, left atrium; LV, left ventricle; PA, pulmonary artery; RA, right atrium; RV, right ventricle; SVC, superior vena cava. Panel B shows the method of left ventricular subendocardial ablation. The umbilical tapes were threaded around the pulmonary arteries and veins, and a catheter was inserted via the left atrial appendage into the left ventricular cavity. A 20F Foley catheter with a stiff guide wire in the central lumen was inserted into the left ventricle. The balloon of the Foley catheter was located roughly at the aortic valve area. The umbilical tapes were tightened to prevent the communication between the pulmonary circulation and the left heart. The balloon on the Foley catheter was then inflated with 25 ml of air to occlude the left ventricular outflow tract. The left ventricular cavity was then flushed with 25–50 ml of Lugold’s solution. After 10–20 seconds, the Lugold’s solution was withdrawn, and the ventricular cavity was irrigated multiple times with warm normal saline. The umbilical tape was released and the balloon of the Foley catheter deflated. The total ischemic time was kept to <2 minutes.

Poles of the S2 stimulating electrode were separated by roughly 3.5 cm, with 56 recording electrodes in between (Figure 1A). The completed electrode array was then applied to the epicardium of the right ventricle.

Surgical preparation. Adult mongrel dogs were anesthetized with sodium pentobarbital (25–35 mg/kg i.v.),8,9 intubated, and ventilated with room air by a Harvard respirator (Harvard Apparatus, Millis, Mass.). An arterial line was inserted into the right femoral artery to continuously monitor systemic blood pressure. Blood was drawn to determine the pH, PO2, PCO2, base excess, and bicarbonate concentrations. Normal metabolic status was maintained throughout the study by taking blood samples every 60 minutes and correcting any abnormal values. Esophageal temperature was continuously monitored and was maintained at approximately 36–37°C by heating the table with warm circulating water. Blood pressure was recorded throughout the study by a Brush Clevitte Mark 200 recorder (Cleveland, Ohio).

The chest was opened through a median sternotomy, and the heart was suspended in a pericardial cradle. The azygos vein was ligated. Umbilical tapes were threaded around the venae cavae and the pulmonary artery in preparation for inflow and outflow occlusion (Figure 2A). The vagus and the phrenic nerves were identified and then separated from the venae cavae. The umbilical tapes passed between the venae cavae and the nerves so that the latter were not included when the tape was tightened. A catheter was inserted via the right atrial appendage into the right ventricular cavity. Two patch defibrillation electrodes with an active surface area of 13.5 cm2 (CPI, St. Paul, Minn.) were sutured to the left and right ventricular epicardia, away from the recording electrodes. The patches were then connected to a defibrillator for electrical defibrillation after each episode of VF. The umbilical tapes around the venae cavae and the pulmonary artery were tightened, and 25–50 ml of warm normal saline was used to flush the right ventricular chambers multiple times (Figure 2A). The occlusion was then released, and the dog was allowed to recover until the blood pressure and the heart rate stabilized. The total duration of inflow and outflow occlusion was <2½ minutes. The recording and pacing electrode array was then placed on the right ventricular anterior wall. Superficial epicardial sutures were used at the edges of the Silastic sheets to pull the tissue flush.
against the undersurface of the recording electrodes. No suture was applied in the area where electrodes were in direct contact with the epicardium. All electrodes stayed at the same location throughout the study.

The recording electrodes were then connected to a Bard electrophysiology computerized mapping system. The bipolar electrogram was filtered from 30 Hz (high pass) to 300 Hz (low pass) and digitized at 1,000 samples per second with 12 bits of precision. Surface ECG leads I, II, III, aVR, aVL, aVF, and V\textsubscript{6} and 56 channels of bipolar intracardiac electrograms were recorded simultaneously. The surface ECG and eight selected intracardiac electrogram signals were also continuously displayed on a monitor throughout the study. A multichannel stimulator was used to drive constant-current stimulation isolation units (Bloom, Reading, Pa.) to give the S\textsubscript{1} stimulation at twice cathodal diastolic threshold and the S\textsubscript{2} stimulation at 0.1–100 mA. The stimulus duration was 5 msec for both the S\textsubscript{1} and the S\textsubscript{2}.

**VF threshold and strength–interval curves.** VF threshold testing was performed by giving a single strong premature bipolar S\textsubscript{1} stimulus\textsuperscript{1,2,10} after the eighth S\textsubscript{1}. The S\textsubscript{1} cycle length was 300 msec. There was a 3-second pause between the S\textsubscript{1} stimulation and the S\textsubscript{2} of the next cycle. The first strength of the S\textsubscript{2} was 10 mA. The whole T wave was scanned with a progressively increasing S\textsubscript{1}–S\textsubscript{2} interval at 10-msec increments until the S\textsubscript{2} fell at the end of the T wave. The S\textsubscript{2} strength was then increased in 2-mA steps until VF was induced. The dog was defibrillated within 10–15 seconds. The same test was performed 5–10 minutes later. If two consecutive tests showed that VF could be induced with stimulus strengths within 4 mA of each other, the test was considered complete, and the average of the two stimulus strengths that induced VF was taken as the VF threshold. We chose 4 mA and not 2 mA (Reference 5) to decrease the time needed for VF threshold determination.

The methods used to determine the strength–interval curves of the anodal and the cathodal stimulations have been described previously.\textsuperscript{11,12} A bipolar S\textsubscript{1} stimulus was introduced at late diastole with an S\textsubscript{1}–S\textsubscript{2} interval of 290 or 300 msec. The first S\textsubscript{1} stimulus strength was 0.1 mA. The strength of the S\textsubscript{2} was then gradually increased until capture occurred. An isochronal map was then generated to determine the site of origin of the evoked activations. If the origin was from the cathodal pole of the bipolar S\textsubscript{2}, then this stimulus strength was the cathodal threshold. The polarity of the S\textsubscript{2} stimulation was reversed, and the origins of the evoked activations were again determined to document that the site of the origin was still cathodal. The polarity was then reversed back to the original combination, and the S\textsubscript{2} strength was progressively increased until the dual origin of the propagated response was observed. This stimulus strength was the anodal threshold. The S\textsubscript{1}–S\textsubscript{2} interval was then progressively decreased, and the same procedure was repeated until the strength–interval curves of the anodal and the cathodal stimulation were determined. The same inflow and outflow occlusion was then released. The total duration of inflow and outflow occlusion was <2½ minutes. After approximately 20–30 minutes of rest, when the blood pressure and the pulse rate had stabilized, the strength–interval curves and the VF threshold were redetermined.

At the end of the study, the dogs were killed by an overdose of pentobarbital. The plaque electrode array was removed, and the tissue underneath it was excised from the rest of the heart. The tissue was then fixed in formalin. Serial sections were taken transmurally to determine the depth of myocardial injury by the Lugol's solution and whether or not the Purkinje fiber network was ablated.

**Protocol 2: Biventricular Subendocardial Ablation**

In protocol 2, biventricular subendocardial ablation was performed. The recording electrode array used in protocol 1 was also used in protocol 2. The locations of the S\textsubscript{1} stimulating electrodes are shown in Figure 1B. To compare the epicardial and systemic temperatures, a temperature probe was embedded in the left ventricular myocardium and another probe was inserted in the esophagus or the rectum.

The recording electrode array was sutured to the left ventricular epicardium, and the VF threshold and the strength–interval curve were determined according to the same method as described in protocol 1. Umbilical tapes were then threaded around the pulmonary arteries and veins (Figure 2B). A catheter was inserted via the left atrial appendage into the left ventricular cavity. The innominate artery was isolated and ligated. A 20F Foley catheter with a stiff guide wire in the central lumen was inserted via the innominate artery retrogradely into the left ventricle. The balloon of the Foley catheter was located roughly at the aortic valve area. The umbilical tapes were tightened to prevent communication between the pulmonary circulation and the left heart. The balloon on the Foley catheter was inflated with 25 ml of air to occlude the left ventricular outflow tract. The left ventricular cavity was then flushed with 25–50 ml of Lugol's solution. After 10–20 seconds, the Lugol's solution was withdrawn, and the ventricular cavity was irrigated multiple times with warm normal saline. The umbilical tape was released and the balloon of the Foley catheter deflated. The total ischemic time was kept to <2 minutes. The Foley catheter was removed, and the dog was allowed to recover for at least 15 minutes. The right ventricular subendocardium was then ablated by the Lugol's solution while the ventricles were paced by a pacemaker. The VF threshold and the strength–interval curves were then redetermined.

**Terminology**

The term "early site" is defined as an electrode location that recorded activation with a shorter latency than all other surrounding electrodes.

The "total activation time" is the time needed for a wave front to activate the mapped tissue and is calculated as the difference between the time of the latest activation and the time of the earliest activation registered in the mapped tissue for any given wave front.

The "duration of the anodal supernormal period" refers to the period when the anodal excitability was higher than expected for a given S\textsubscript{1}–S\textsubscript{2} interval. It starts...
when the decreasing S₁-S₂ interval is associated with a decreasing anodal threshold and ends when a further decrease of the S₁-S₂ interval results in either noncapture or the elevation of the anodal threshold beyond the previous highest value.

Data Analysis

The recordings from each channel were displayed on a computer terminal. Baseline sinus activation and the patterns of activation after the S₁ and the S₂ stimulation were determined. The time selected for activation was the fastest slope on the electrogram. Isochronal activation maps were drawn for all complexes analyzed.

Student's t test and ANOVA were used to analyze the difference of the means. The null hypothesis was rejected for values of p<0.05. The paired t test was also used to compare the late diastolic anodal threshold with the late diastolic cathodal threshold and to compare the late diastolic anodal threshold with the anodal threshold in the supernormal period. Because the late diastolic anodal threshold was used twice, values of p had to reach ≤0.025 to be considered significant (Bonferroni procedure).

Results

Protocol 1: Right Ventricular Subendocardial Ablation

Eight dogs underwent right ventricular subendocardial ablation, and six survived. These six dogs weighed 18±3 kg (mean±SD); their hearts weighed 162±32 g. Although three of the six dogs required manual cardiac massage for up to several minutes immediately after irrigation with Lugol’s solution, none received atropine, epinephrine, or other sympathetic stimulants for resuscitation.

Physiological evidence supporting the effective ablation of the Purkinje fibers and the subendocardial myocardium in the right ventricle. In each dog, right bundle branch block developed immediately after Lugol’s solution was introduced into the right ventricular cavity. The patterns of activation at baseline and after normal saline irrigation (Figure 3) were the same, with the earliest site of activation located at the left and lower portion of the electrode array, which is compatible with the normal epicardial activation sequence. After irrigation with Lugol’s solution (Figure 4), right bundle branch block occurred, and the early site shifted from the left lower edge of the mapped tissue to the left upper edge, indicating that the wave front was now originating from the left ventricle. With the onset of surface QRS as time zero, the time of activation at the early site increased from 11±4 msec before ablation to 42±8 msec after ablation (p<0.0001).

Figure 5 shows the epicardial activation patterns during stimulation at the cathodal diastolic threshold before (panel A) and after (panel B) subendocardial ablation. In both panels, the spread of activation started from the cathodal site, indicating that the cathodal threshold was lower than the anodal threshold. However, the total activation time was longer after ablation than before ablation. Figure 6 shows that, during the supernormal period, activation started from the anodal site. The total activation time was also longer after ablation than before ablation.

Table 1A shows the effects of subendocardial ablation on the total activation time of the mapped tissue. Subendocardial ablation significantly increased the total activation time during sinus rhythm, at the cathodal late diastolic threshold, and during the anodal supernormal period.

In Figures 5 and 6, the spread of excitation before and that after subendocardial ablation were similar near the site of capture. Beyond the first several rows of the recording electrodes, however, the spread of excitation was dramatically different. The spread of excitation before ablation was characterized by almost simultaneous epicardial activation over a large area (between isochronal lines 40 and 50 in Figure 5A and between isochronal lines 70 and 80 in Figure 6A). After subendocardial ablation, such a phenomenon was no longer observed, and the epicardial isochronal lines were close together away from the early site as they were when adjacent to the early site (Figures 5B and 6B).
myocardial fibers with granularity of the cytoplasm was obvious. Vacuolation of the fibers occurred (Figure 7C), leaving a peripheral rim of sarcoplasm with a displaced nucleus and myocytolysis. Fragmentation and coagulative necrosis were also present. There was a more intense eosinophilic staining of the cytoplasm (Figure 7A). Early contraction band necrosis with intense eosinophilic transverse bands spanning the myofiber was minimal. There was no evidence of a marked neutrophilic, macrophage, or fibrovascular response.

In four of the eight dogs studied, one or more of the Purkinje fibers still possessed a nucleus. However, partial necrosis is evident in each of these cells (Figure 7D). It is highly likely that these cells sustained a significant degree of injury. We did not observe any morphologically normal Purkinje fibers in the histopathological sections.

**Anodal supernormality before and after subendocardial ablation.** Although the anode and the cathode of the S1 were separated by 3.5 cm, simultaneous determination of the anodal and the cathodal strength–interval curves was possible because the interval from the onset of the S0 stimulation to the local activation at the electrode closest to the anodal site was not significantly different from that for the cathodal site. Before subendocardial ablation, the times when the S1 wave front reached the anodal and cathodal S1 sites were 43±8 and 43±10 msec, respectively (p=NS). After subendocardial ablation, the times when the S1 wave front reached the anodal and cathodal S1 sites were 45±7 and 44±9 msec, respectively (p=NS). Assuming that the repolarization sequence was the same as the depolarization sequence, the anodal and the cathodal sites should be at roughly the same stage of repolarization when the S1 was given.

The cathodal and the anodal late diastolic thresholds were 0.35±0.19 and 0.98±0.26 mA, respectively (p=0.004) after irrigation with normal saline and were 0.53±0.49 and 1.00±0.44 mA, respectively (p=0.004) after subendocardial ablation with Lugol’s solution. Therefore, the propagated wave front always originated from the cathodal site during late diastole. Reversing the polarity of the S1 resulted in reversing the site of origin of the wave front in all dogs. Because reversing the polarity of the S1 resulted in reversal of the site of capture, the demonstration of a lower cathodal than anodal stimulation threshold was a result of a true difference of threshold rather than of the different extent of repolarization after the S1 stimulation at the two sites.

Figures 8A–8F show the strength–interval curves of the six dogs studied in protocol 1 before chemical ablation of the right ventricular subendocardium. Consistent with previous observation,11 the cathodal threshold increased as the S1–S2 interval progressively decreased. Although in three dogs the decrease of the S1–S2 interval was associated with a transient decrease of the cathodal threshold (panels C, E, and F), the changes were small (≤0.5 mA) and the duration was brief (10 msec). The anodal curve, on the other hand, did not rise smoothly but showed an apparent period of relative supernormality (arrows). After subendocardial ablation, the anodal supernormal excitability persisted (Figures 9A–9F).

At baseline, the anodal threshold at late diastole was not significantly higher than the anodal threshold during

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**Figure 4.** ECGs and map showing patterns of right ventricular epicardial activation during sinus rhythm after irrigation with Lugol’s solution. Panel A shows that, after the application of Lugol’s solution, a right bundle branch block pattern immediately developed. Panel B shows the actual recordings of selected electrodes in column F and the surface ECG aVF. Vertical line indicates the onset of the QRS complex. Epicardial activation (panel C) initiated from the left upper aspect of the mapped tissue. The time at the early site (arrow) also increased from 10 to 35 msec, compatible with the delayed activation of the right ventricle caused by right bundle branch block. Total activation time increased from 33 msec in Figure 3C to 48 msec in panel C of this figure, compatible with successful ablation of the Purkinje fiber network.

This is an additional piece of evidence that the subendocardium and the Purkinje fibers were effectively ablated by this technique.

**Histopathological evidence.** In all, 71 serial transmural sections were examined for the eight dogs studied (9±2 sections per dog). In all dogs, the Purkinje fibers and the subendocardial contractile myofibers showed changes consistent with early necrosis. The Purkinje fibers normally differ from the contractile fibers in that Purkinje fibers are larger, contain fewer myofibrils, and possess a greater quantity of clear cytoplasm containing increased glycogen. The layer of necrotic subendocardial myocardial cells approximated a zone of up to six or seven myocardial cells (Figures 7A and 7B). No Purkinje fibers were observed outside the zone of necrosis. There was a slight separation of the Purkinje and the contractile fibers by edematous fluid and erythrocytes that had seeped in from the capillaries into the interstitium. Both stretching and waviness of the fibers were present, a condition caused by the systolic tug of the viable fibers adjacent to the necrotic fibers. Cloudy swelling of the
the supernormal period (0.98 ± 0.26 versus 1.18 ± 0.47 mA, p = NS). After subendocardial ablation, the anodal threshold at late diastole was also not significantly different from the anodal threshold during the supernormal period (1.00 ± 0.44 versus 0.90 ± 0.28 mA, p = NS).

Before and after subendocardial ablation, the longest S1–S2 intervals associated with an anodal supernormal period were 188 ± 21 and 188 ± 13 msec (p = NS), the lowest anodal thresholds associated with the supernormal period were 0.98 ± 0.26 and 1.00 ± 0.44 mA (p = NS), and the durations of the anodal supernormal periods were 28 ± 10 and 27 ± 16 msec, respectively (p = NS). Thus, subendocardial ablation did not significantly alter the timing, magnitude, or duration of the supernormal period.

VF threshold before and after ablation. The VF thresholds were not significantly different before and after chemical ablation of the subendocardium of the right ventricle (Table 2A).

Protocol 2: Biventricular Subendocardial Ablation

Biventricular subendocardial ablation was performed in 11 dogs. Among them, seven dogs died during the procedure. Five of the seven dogs died immediately after the left ventricular subendocardial ablation, probably because of failure to properly occlude the left ventricular outflow tract when Lugol’s solution was injected into the left ventricular cavity. Two dogs died of progressive hypoxia and hypotension after initial success in left ventricular subendocardial ablation. Four dogs survived biventricular subendocardial ablation and completed the study protocol. Each of the four dogs developed complete heart block after biventricular subendocardial ablation. The dogs weighed 23 ± 4 kg, and their hearts weighed 204 ± 6 g. In only one dog was the blood pressure unchanged after ablation. In the other three dogs, the systolic blood pressure decreased 35–60 mm Hg after biventricular ablation. In each dog, the temperature measured in the myocardium was approximately the same as that measured in the esophagus or rectum, with a maximal difference of 0–2°C. The body temperature was 35°C or higher in three dogs and was 32°C in dog 10. The temperatures were similar before and after ablation in each dog.

Evidence supporting the effective ablation of the Purkinje fibers and the subendocardial myocardium in the left ventricle. Two transmural sections were obtained from the right and left ventricular free wall of each dog. Microscopic examination of the subendocardium of both ventricles revealed necrosis of the Purkinje fiber network and the subendocardial myocytes. The changes were essentially the same as those shown in Figure 7.

Figure 10A shows that, before subendocardial ablation, all dogs had a narrow QRS complex. The epicardial activation patterns on the left ventricle (Figures
TABLE 1. Effects of Subendocardial Ablation on the Total Activation Times

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<th>After normal saline</th>
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<td>Sinus rhythm</td>
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<td>At anodal supernormal period</td>
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FIGURE 6. Maps showing patterns of right ventricular epicardial activation during the anodal supernormal period before and after subendocardial ablation. Examples are from dog 1. Panel A shows that, after irrigation with normal saline, an S2 of 0.7 mA given 180 msec after the last S1 resulted in capture near the anodal site (arrow), with a latency of 42 msec. Total activation time was 41 msec. Panel B shows that, after subendocardial ablation with Lugol’s solution, an S2 of 0.8 mA given 190 msec after the last S1 resulted in capture near the anodal site (arrow), with a latency of 43 msec. Total epicardial activation time was 59 msec. The increased total activation time is compatible with successful ablation of the Purkinje fiber network. ☃️, Cathode; ⚡️, anode; ⛔️, site at which adequate recording was not obtained.

10B and 10C) showed rapid activation of the entire mapped epicardium within 12 msec. After subendocardial ablation, left bundle branch block developed immediately (Figure 11A). The total epicardial activation time increased to 47 msec (Figures 11B and 11C), indicating that propagation of the activation wave front was via the myocardial cells and not the subendocardial Purkinje fibers. Table 1B shows the effects of subendocardial ablation on the left ventricular epicardial total activation times. The increased total activation times indicate successful subendocardial ablation.

Anodal supernormality before and after subendocardial ablation. Before subendocardial ablation, the times when the S1 wave front reached the anodal and cathodal S2 sites were 47±15 and 48±16 msec, respectively (p=NS). After subendocardial ablation, the times when the S1 wave front reached the anodal and cathodal S2 sites were 49±19 and 51±19 msec, respectively (p=NS). Assuming that the repolarization sequence was the same as the depolarization sequence, the anodal and the cathodal sites should be at roughly the same stage of repolarization when the S2 was given.

Panels G-J of Figure 8 show the strength–interval curves of the four dogs before biventricular subendocardial ablation. Anodal supernormality (arrows) was demonstrated. Panels G-J of Figure 9 show the strength–interval curves after subendocardial ablation. Anodal supernormality was still present.

The anodal and the cathodal late diastolic thresholds were 1.38±0.36 and 0.30±0.14 mA, respectively (p=0.009) at baseline and were 1.03±0.52 and 0.25±0.06 mA, respectively (p=NS) after subendocardial ablation with Lugol’s solution. At baseline, the anodal threshold at late diastole was not significantly higher than the anodal threshold during the supernormal period (0.50±0.18 mA, p=NS). After subendocar-
The anodal threshold at late diastole was also not significantly different from the anodal threshold during the supernormal period (0.53±0.21 mA, p=NS). Before and after subendocardial ablation, the longest S1–S2 intervals associated with the anodal supernormal period were 218±25 and 220±18 msec (p=NS), the lowest anodal thresholds associated with the supernormal period were 0.50±0.18 and 0.53±0.21 mA (p=NS), and the durations of the anodal supernormal period were 23±5 and 25±17 msec (p=NS), respectively. Thus, subendocardial ablation did not significantly alter the timing, the magnitude, or the duration of the supernormal period.

**Discussion**

**Effects of Chemical Ablation of the Subendocardium**

In normal healthy dogs with heart in situ, chemical ablation of the right ventricular subendocardium alone or of the subendocardium of both ventricles did not decrease ventricular vulnerability to single premature electrical stimulation. This negative result differs from what has been obtained by other investigators. Why are our results so different? One explanation could be that the method we used does not result in the effective ablation of the subendocardial tissue near the site of the electrical stimulation. We believe that this is not the case. During the study, 25–50 ml of Lugol's solution was injected into the ventricular cavity, resulting in the apparent distension of the injected ventricle, and there was often a backflush of the solution around the atrial entry site of the catheter. This method should result in a more complete subendocardial ablation than is obtained by painting, because the trabeculation of the ventricular subendocardium might prevent the brush or cotton swab from touching the invaginated areas between the trabeculae. Thus, we believe that our method was at least as effective as painting in ablating the Purkinje fiber network. This conclusion is supported by the mapping studies of the activation sequences as well as by the histopathological studies. Furthermore, the method of ablating the subendocardium in our study

**Figure 7.** Facing page. Histopathological changes after right ventricular subendocardial ablation with Lugol’s solution. In panel A, the large, vacuolated cells are the necrotic Purkinje fibers (PNEC). Eosinophilic staining of the myocardium (EO) is observed. Cloudy swelling with granularity of the cytoplasm was commonly observed (CL&GR), as was pyknosis (PYK). There is separation of the Purkinje cells and the contractile fibers by edematous fluid and erythrocytes. Both stretching and waviness (W) of the fibers are present. The layer of necrotic subendocardial myocardial cells approximated a zone of five myocardial cells, which roughly equals a depth of 0.5 mm from the endocardium. The rest of the myocardial cells are normal (NL). Original magnification, ×200. Panel B shows a high-power view of the necrotic Purkinje cells. The large, vacuolated cells are the necrotic Purkinje cells. Edema is present in the subendocardium, with a few layers of necrotic myocardial cells. The cells in the lower part of the figure are normal. Original magnification, ×400. Panel C shows vacuolation of the subendocardial myocytes. Arrow points to a subendocardial myocyte that demonstrates vacuolation. There was separation of the Purkinje and the contractile fibers by edematous fluid and erythrocytes that had seeped in from the capillaries into the interstitium. Original magnification, ×600. Panel D shows possible viable Purkinje fibers. Arrow points to a Purkinje fiber that retains a nucleus, which is a histological characteristic of viability. However, partial necrosis of this fiber is also evident. All other Purkinje fibers in this section show coagulation necrosis without nuclei. Original magnification, ×600.

**Figure 8.** Strength–interval curves before chemical ablation of the subendocardium. Based on the origin of impulse determined by the isochronal maps, the strength–interval curves of each animal can be constructed for anodal and cathodal stimulation. Panels A–J show the strength–interval curves of dogs 1–10, respectively. Among them, dogs 1–6 belong to protocol 1 and dogs 7–10 to protocol 2. The filled and unfilled circles represent the anodal and cathodal stimulations, respectively. In all dogs, a relatively supernormal period was demonstrated on the anodal strength–interval curve (arrows). RV, right ventricle; LV, left ventricle.
FIGURE 9. Strength–interval curves after chemical ablation of the subendocardium with Lugol’s solution. Panels A–J show the strength–interval curves of dogs 1–10, respectively. Among them, dogs 1–6 belong to protocol 1 and dogs 7–10 to protocol 2. The filled and the unfilled circles represent the anodal and the cathodal stimulations, respectively. There is a persistence of anodal supernormality (arrows) despite subendocardial ablation. RV, right ventricle; LV, left ventricle.

does not require ventriculotomy and hence avoids myocardial injury.

A second consideration is the fact that we studied the in situ hearts of normal healthy dogs. Compared with studies using isolated tissue preparations or isolated, Langendorff-perfused hearts, the in situ heart is still innervated and is influenced by humoral autonomic stimulation. The ventricles were also contracting against the pulmonary or systemic vascular resistance. It was demonstrated that alteration of the mechanical status of the myocardium can affect the repolarization process of the cardiac myocytes. Cardiopulmonary bypass, which unloads the ventricles, was found to produce substantial and immediate prolongation of the action potential. Furthermore, mechanoelectrical feedback was shown to affect the electrical induction of ventricular arrhythmia, at least in the infarcted canine model. Previous studies either used isolated heart preparation or obtained their data when the dogs were on cardiopulmonary bypass with empty ventricular cavities. Because these ventricles were not contracting against a normal vascular resistance, mechanoelectrical feedback might have affected the VF threshold testing.

From these analyses of previously published work, it can be seen that significant differences in methods exist among different investigators. These differences in method might at least partially explain the different results that were obtained. However, our study using intact animals with normally contracting ventricles at least raises the question of how effective chemical ablation of the subendocardium is as a method to prevent the generation and the maintenance of VF.

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<th>Table 2. Ventricular Fibrillation Threshold</th>
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<td>Mean±SD*</td>
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VFT₁, first ventricular fibrillation threshold determined; VFT₂, second ventricular fibrillation threshold determined; VFT, average of VFT₁ and VFT₂. *There was no statistically significant difference between these two means.
Clinical trials should not be performed until more studies of intact animals show the efficacy of this method and document the mechanisms by which subendocardial ablation prevents the induction of VF.

**Supernormal Excitability and the Purkinje Fibers**

It is uncertain whether or not the supernormal excitation observed in the intact animals\(^6,\)\(^7,\)\(^10,\)\(^11,\)\(^28\) was caused by stimulation of the nearby Purkinje fibers or by stimulation of the ventricular myocytes. In one experiment using isolated Purkinje fiber–papillary muscle preparations, supernormal excitation was demonstrated only in the Purkinje fibers and not in the subendocardial ventricular myocytes. These results prompted the authors to hypothesize that the supernormal excitability of the ventricular myocardium that is observed when surface stimulation is used may be caused by the stimulation of the adjacent Purkinje fibers. In this study, we demonstrated anodal supernormal excitation both before and after chemical subendocardial ablation. There are at least three explanations for these findings. One explanation is that, despite the fact that no surviving Purkinje fibers were identified by histopathological and light microscopic examination, some Purkinje fibers might have survived. Without an electron microscope, differentiating between the Purkinje fibers and the contractile myocytes may be difficult.\(^29,\)\(^30\) Spach et al.\(^31\) also reported that they were able to record Purkinje potentials up to 3 mm beneath the endocardial surface in the left ventricular free wall and up to 1 or 2 mm in the right ventricular free wall. The subendocardial cell necrosis produced by topical application of Lugol's solution measured only 0.5 mm in depth.\(^3\) Thus, some Purkinje fibers might have survived the ablation and served as the source for supernormal excitation.\(^7\)

The second possible explanation of the discrepancy between our results and those obtained by others? can be explained by the presence of electrophysiological heterogeneity within the ventricular wall.\(^3\) The epicardial cells and the subepicardial M cells have a prominent transient outward current with slow reactivation kinetics, a fact that may contribute to the presence of a supernormal phase of excitation and conduction in the ventricular myocardium.\(^3\) Because prominent transient outward current was not present in the endocardial cells, studies using isolated endocardial myocytes\(^7\) may thus not demonstrate supernormal excitation.

The third explanation is the different methods used in determining the supernormal excitability. The supernormal excitability described in previous studies\(^6,\)\(^7\) was demonstrated by intracellular current injection. Because we could not perform intracellular current injection in a beating heart, extracellular stimulation technique was used in this study. The different methods used to demonstrate supernormal excitability may explain the different results observed.

**Limitations of the Study**

One limitation of this study is the method used in determining the anodal and the cathodal strength–interval curves. At the beginning of this study, we were concerned about the physiological stability of the dog

**FIGURE 10.** ECGs and map showing epicardial activation patterns of the left ventricle before subendocardial ablation. Example was taken from dog 7. Panel A shows a normal ECG with a narrow QRS complex. Panel B shows the actual recordings of selected electrodes in column F and the surface ECG aVF. Panel C shows the isochronal activation map with the onset of the QRS complex (vertical line in Panel B) as time zero. Arrows point to the early sites. Isochronal lines are 10 msec apart. The whole epicardium was activated within 12 msec. These are normal activation patterns. •, sites at which adequate recording was not obtained.
after inflow and outflow occlusion and irrigation with Lugol's solution. If the anodal and the cathodal strength-interval curves are determined at different times, physiological instability may render the results unreliable. The method we used in this study has the advantage of determining both curves simultaneously and thus is less dependent on the stability of ventricular excitability over time. The method has also been well reported in the literature. The disadvantages of this method are that the conduction time between the S1 and the S2 sites may vary and that the strength–interval curves for the anode and the cathode were determined at different ventricular sites. Nevertheless, the anodal supernormality was clearly demonstrated both before and after irrigation with Lugol's solution, indicating that the method used did not affect our ability to detect a supernormal period.

A second limitation is that, after left ventricular subendocardial ablation, the systemic blood pressure often significantly decreased. This phenomenon could have affected the results of this study. However, there were no significant changes in the electrophysiological parameters—such as the timing, the duration, and the magnitude of the supernormal period—before and after subendocardial ablation. Furthermore, none of the four dogs that survived the procedure were supported by sympathetic stimulants at any time during the study. Thus, we doubt that hypotension significantly affected the results of this study. However, hypotension may make the chronic animal studies difficult. It is interesting to note that, despite the highly promising results, none of the other investigators have subsequently reported experiments using chronic animal models to document the antifibrillatory effects of subendocardial ablation. Thus, long-term survival after biventricular subendocardial ablation has not been documented by experimental studies. This fact further supports our contention that clinical tests should not be performed until more is known about this procedure.

Acknowledgments
The authors wish to thank Richard Pavelec, Amy Bloom, Dhiraj Bhatia, and Dustin Hough for their technical assistance and Kerry Hunter for her secretarial assistance.

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P S Chen, P L Wolf, Y M Cha, B B Peters and S L Topham

_Circulation_. 1993;87:216-229
doi: 10.1161/01.CIR.87.1.216

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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